Detection of CD-40 in Frozen Mouse Tissue

Reagents:

1X Automation Buffer
3% Hydrogen Peroxide
Antibody Diluent
Citrate Buffer
DAB Chromagen
Hematoxylin

Antibody Information

Blocking serum: Normal Goat Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #005-000-121

Avidin Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #SP-2001

Negative control: Purified Rat IgG 2a
BD Pharmingen
Distributed by Transduction Labs
Lexington, KY 40511
1-800-227-4063
Catalog # 559073

Primary antibody: (Rat anti-mouse CD40 monoclonal)
BD Pharmingen
Distributed by Transduction Labs
Lexington, KY 40511
1-800ó227-4063
Catalog # 550285

Secondary Antibody: Biotin-Conjugated Goat anti-rat IG (muliple adsorbed)
BD Pharmingen
Distributed by Transduction Labs
Lexington, KY 40511
1-800ó227-4063
Catalog #5590286
Suggested dilution 1:200

Label antibody:Super Sensitive Label Antibody

Biogenex Laboratories

San Ramon, CA 94583

www.biogenex.com

1-800-421-4149

Catalog #HK330-5K

Staining Procedure

-Positive Control Tissue: Mouse spleen and thymus (B-cells and dendritic cells)

-Stain localization: Cell membrane

For Frozen Tissue Sections

Two sequential 6 micron sections were cut per slide (Probe-On Plus by Diagger). Sections are cut and immediately fixed in Rapid Fix (Shandon-Lipshaw) for 7 seconds. Place section in 1X AB. After the last section is cut, wash in 1X AB for 5 minutes. Repeat buffer wash.

Cut sections the day of staining.
Allow to air dry 30 minutes at room temperature after the last slide has been cut.
Place slides in cold acetone (-20) for 2 minutes.
Allow slides to air dry for 30 minutes.
Place in 1X AB for 5 minutes

1 Quench endogenous peroxidase by placing slides in 0.3% hydrogen peroxide for 30 minutes.

2 Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

3 Apply 5% Normal Goat Serum for 20 minutes at room temperature.
Lot#_____ Reconstituted Date_____

Wipe excess reagent from around tissue section. DO NOT RINSE SECTIONS WITH BUFFER.

5 Apply Avidin/Biotin block
Lot#_____ Exp Date_____ New kit yes / no
Apply avidin block - 15 min @ RT.
Quick rinse in 1X AB.
Apply biotin block - 15 min @ RT.

No wash, wipe excess block and apply primary antibody

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

6. Apply primary antibody (CD-40) at a 1:40 dilution and incubate for one hour.
Lot#_____ Aliquoted yes / no Date Aliquoted_____

On negative control slides, normalize the concentration of purified Rat IgG-2a negative control with the protein concentration of the CD-40 antibody. Apply to slides at a 1:40 dilution and incubate for one hour.
Lot#_____ Reconstituted Date_____

7. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

8. Apply secondary antibody (Goat anti-rat IgG) at a 1:200 dilution and incubate for 30 minutes.
Lot#_____ Reconstituted Date_____

9. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

10. Apply Label antibody (StriAviGen Super Sensitive Predilute) for 30 minutes.
Lot #_____ Exp. Date _____

11 Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

12 Apply liquid Dako DAB Chromagen for 6 minutes in the dark.
(Add 1 drop of DAB per ml of substrate)
Lot#_____ Exp. Date_____ new kit yes / no

13 Rinse in tap water 3 minutes.

14 Counterstain with Modified Harris Hematoxylin for 30 seconds.

15 Rinse in tap water until water is clear.

16 Place slides in 1X Automation buffer for one minute with gentle agitation to blue slides.

17 Dehydrate through the following solutions.

95% alcohol	1 times	3 mins
100% alcohol	3 times	3 mins
Xylene	2 times	5 mins

18. Coverslip.
updated 1/14//2004